Determination of the effects of dietary probiotic and environmental enrichment on weaning pig growth performance and measures of oxidative stress.

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Abstract

Weaning introduces a multitude of stressors that negatively impact feed intake and can diminish gastrointestinal (GI) integrity which leads to increased disease susceptibility and altered gut function. Probiotics have been studied as an alternative to antibiotic growth promoters to enhance gut health. Furthermore, environmental enrichment has been shown to reduce the time spent in negative social interactions and increase feed intake in a stressful environment. The objective of this study was to determine effects of dietary probiotic (*Saccharomyces cerevisiae*) supplementation on weaning pig growth performance and oxidative stress, with and without environmental enrichment. The experiment was a 2 x 2 factorial design with 2 dietary treatments fed in nursery pens that did or did not include environmental enrichment. Weaned piglets (n=100 and 5pigs/pen; ~25.6 day of age) were allotted to the following dietary treatments: 1) control or 2) yeast probiotic *Saccharomyces cerevisiae* (0.1% in phase 1 and 2 and 0.05% phase 3 diets) for 5-wks. On days 1, 7, 14, 21 and 35, pig weights, feed intake, fecal scores and blood samples were collected for monitoring diarrhea and measurement of oxidative stress markers. Pen weight (kg) was significantly increased over the five-week period. Pigs fed the basal diet had greater ADG (g) compared to pigs fed the probiotic supplemented diet (overall weeks 460.51 g vs 424.89 g, respectively $P < 0.06$). Pigs fed the probiotic diet tended to have more solid stool than pigs on the basal diet ($P < 0.15$). Oxidative stress marker diamine oxidase (DAO) fluctuated over the first 14 days of the nursery trial. There was a significant day effect from day 1, 7 and 14 post-weaning DAO (26.5, 18.3, and 22.1 ±1.4mg/mL respectively; $P < 0.0001$). In conclusion, probiotic supplementation did not enhance growth performance, but did tend to have improved stool consistency. The environmental enrichment used did not significantly impact growth or systemic oxidative stress. Overall, dietary supplementation and environmental enrichment did not improve weaning pig growth performance and oxidative stress markers in the study.

Introduction

Weaning presents simultaneous nutritional, environmental, and psychological stressors. These stressors culminate in a post-weaning growth check that results in a disturbance of gastrointestinal (GI) health (Moeser, 2017). The stress also coincides with a decline of passive immunity from sow milk and a critical window of GI development. In-feed antibiotic growth promoters have been used to reduce the impact of weaning stress and optimize efficiency of production, but consumer demand and legislation limiting their use leaves the swine industry on the search for alternatives to maintain animal welfare and optimize feed efficiency (Liao, 2017).

Nutritional interventions that optimize GI health will optimize piglet performance and welfare. Probiotics are a nutritional intervention currently investigated to improve GI health at weaning because of their potential to provide multiple mechanisms to improve intestinal function and animal well-being (Pais et al., 2020). Yeast probiotics have been shown to improve to have the potential to alleviate some postweaning problems (Badia et al., 2012; Pluske et al., 2013; Kiros et al., 2018). Yeast products containing Saccharomyces cerevisiase are good sources of enzymes, nutrients and growth factors utilized by commensal microbes to create a homeostatic intestinal microbiome (Pluske et al., 2013; Kiros et al., 2018). Furthermore, the changes to the intestinal environment observed when offering probiotics seem to enhance intestinal health and produce positive production responses in piglets (Pluske et al., 2013). Since weaning stress is also intensified by fighting and establishing a new social hierarchy, a multitiered approach may be beneficial for animal well-being. Environmental enrichment in production environment has been shown to reduce the time spent in negative social interactions and increase feed intake (Beattie, 2000). Nutritional and management strategies could provide additive effects at weaning to improve animal health and well-being. The objective of this research project was to determine the effects of the dietary probiotic (*Saccharomyces cerevisiae*) supplementation on weaning pig growth, performance, and oxidative stress, with and without environmental enrichment. We hypothesized that the additive effects of the probiotic would improve the health and welfare of the piglet at weaning and provide an acceptable substitute for growth promoting antibiotics currently being used within the swine industry.

Methods

Animals and Diet.

One-hundred weaned piglets (~26 d old) were sorted such that treatment was balanced for litter origin, weight, and sex. Piglets were housed in an environmentally controlled nursery room with *ad libitum* access to feed and water at all times. Piglets were allotted to one of four treatments in a 2 x 2 factorial arrangement and 1) control diet, no enrichment 2) control diet, enrichment 3) yeast diet, no enrichment 4) yeast diet, enrichment. Piglets were housed in 20 pens, with 5 piglets per pen, and 5 pens per treatment. Piglets were fed a standard corn/soybean base nursery diet with three phases associated with intestinal development (Table 1). Yeast supplementation was added to diets at 0.1% in phase 1 and 2, and 0.05% in phase 3. Feed and performance data were collected on days 0, 7, 14, 21, 28, and 35. Pen fecal scores were collected on days 0, 7, 14, 21, 28, and 35. Fecal scores were based on a scoring system (0, dry, hard, well-formed feces; 1, soft, but formed feces; 2, pasty feces green or brown in color; 3, viscous feces light in color, episodic; 4, fluid feces in light color; 5, watery feces, continuous).

Blood collection and serum analysis of diamine oxidase.

On days 1, 7, 14, 21 and 35, blood samples were collected for serum measurement of oxidative stress marker, diamine oxidase (DAO). Serum DAO was measured using a 96-well plate enzymatic assay adapted from Lui et al. (2009).

Statistical Analysis

Individual pens served as the experimental unit. Growth data, weigh, fecal score, and DAO measurements were analyzed as a mixed model with repeated measures in time (week) using the PROC MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The model included fixed effects of diet, enrichment, and week. Random effect was the pen placement. Various covariate structures of error were fitted, and compound symmetry was selected based on the lowest Bayesian Information Criterion (BIC).

Results

Growth Performance

The effect of the nursery diet treatment and enrichment effects are analyzed (Table 2). Pen weight (kg) was significantly increased over the five weeks of the trial as expected ($P < 0.0001$). There were interactions, diet by week and enrichment by week ($P < 0.05$) that contributed to increase pen weights in the basal diet compared to probiotic fed pigs, and enrichment increased pen weights in week five compared to no enrichment pens. The ADFI for the diet by week interaction was significant ($P = 0.026$) with greater feed intake in the basal fed pigs compared to the probiotic supplemented fed pigs. Regarding the enrichment by week interaction, ADFI ($P = 0.003$) was increased in the presence of enrichment after the first week in the nursery. Pigs fed the basal diet had greater ADG compared to pigs fed the probiotic supplemented diet (overall weeks 460.51 g vs 424.89 g, respectively $P < 0.06$). Furthermore, ADG showed an enrichment by week interaction (*P* = 0.053) due to improved ADG in weeks four and five when enrichment was present in the pens.

Stool Scores and Markers of Oxidative Stress

Pigs fed the probiotic diet tended to have more solid stool than pigs on the basal diet ($P < 0.15$). The effect of day was highly significant with days 7, 14 and 28 having most runny stool ($P < 0.0001$). Oxidative stress marker diamine oxidase (DAO) fluctuated over the first 14 days of the nursery trial. There was a significant day effect from day 1, 7 and 14 post-weaning DAO (26.5, 18.3, and 22.1 ±1.4mg/mL respectively; $P < 0.0001$).

Discussion

Overall, dietary probiotic supplementation in pigs weaned 26 days of age with or without environmental enrichment did not improve overall weaning pig growth performance and oxidative stress markers. Better growth performance parameters were anticipated as a result of the additive effects of probiotic supplementation and environmental enrichment. The results in the pen weight, ADFI, and ADG implicate that the probiotic, in this case, was not influential on growth performance, and therefore, nursery performance would not benefit from this type of supplementation. This may be due to yeast probiotics not being able to confer its health benefits in pigs weaned at older ages. Weaning from days 18-21 days of age have previously shown added benefits in growth performance for pigs supplemented with yeast probiotics (Tellman et al., under review). Therefore, it may be beneficial that yeast probiotics are supplemented for pigs weaned at younger ages or even before weaning during farrowing. This would allow for the probiotic to be present in the gastrointestinal tract before various weaning stressors impact animal health and wellbeing.

Oxidative stress measured through serum diamine oxidase was used as a marker for systemic stress within the body. Overall, probiotic use from this study was not seen to have an effect on serum diamine oxidase levels but did have a positive effect on diarrhea scores. Enrichment did have slight significance for improving ADFI and ADG. Therefore, it could be researched further to determine its possible positive effects in a nursery environment. Future studies warrant a look into how weaning age effects on probiotic and antibiotic performance. Depending on the results, it may be more beneficial to the producer to propose a later weaning age, rather than growth promoter supplementation in the proper environment.

Table 1: Calculated Diet Table

	Phase 1	Phase 2	Phase 3
Weight of Pigs	12 -15 lb	15-25 lb	25-50 lb
Ingredient			
Corn	718	1001	1206
Soybean Meal, Dehull, Sol Extr	380	535	685
Bovine Blood Plasma	80		
Corn DDGS, >6 and <9% Oil	100		
Fish Meal Combined	50		
Milk, Whey Powder	500	200	
Choice White Grease	60	40	40
Calcium phosphate (monocalcium)	15	21	23
Limestone, ground	15.5	22	20
Sodium chloride	6	6	7
L-Lys-HCL	4.5	6	6
DL-Met	2.8	3.2	2.5
L-Thr	1.5	2.2	2.3
Trace mineral premix	3	3	3
Vitamin premix without phytase	5	5	5
Choline chloride 60%	0.7		
HiPhos 2700		0.3	0.3
Zinc oxide	7.8	5	
HP 300 (Hamlet Protein)	50	150	
TOTAL	2000.0	2000.0	2000.0
Required SID Lys:NE Ratio	5.65	5.48	5.04
Calculated SID Lysine Required, %	1.47	1.37	1.25

Table 2: Effect of dietary supplementation of yeast probiotics with environmental enrichment on pig performance. Data are presented as means ± SEM for pen weight, average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F). *P*-values for the main effects of diet, enrichment, time as week and the interactions are reported.

Diet	Basal		Probiotic		SEM	P values					
Enrichment	-	+	-	+		Diet	Enrichment	Week	Diet*Enrich	Diet*Week	Enrich*Week
Pen wt, kg						0.322	0.624	< 0.0001	0.583	0.008	0.006
Week 1	45.47	44.16	44.80	43.94	4.40						
Week 2	55.93	56.07	51.91	55.24	4.40						
Week 3	75.15	73.58	68.26	71.71	4.40						
Week 4	93.80	94.70	86.09	92.57	4.40						
Week 5	120.74	121.37	107.70	117.12	4.40						
ADFI, g						0.297	0.406	< 0.0001	0.582	0.026	0.003
Week 1	315.74	257.83	258.33	276.52	55.05						
Week 2	411.36	451.70	383.78	493.79	55.05						
Week 3	647.44	666.88	562.36	566.87	55.05						
Week 4	873.47	951.19	839.66	889.10	55.05						
Week 5	1072.99	1050.26	954.75	1051.64	55.05						
ADG, g						0.06	0.406	<0.0001	0.538	0.270	0.053
Week 1	142.01	106.77	189.05	101.07	39.22						
Week 2	298.80	340.26	199.61	322.90	39.22						
Week 3	548.88	500.16	490.51	470.62	39.22						
Week 4	533.07	603.56	533.59	596.05	39.22						
Week 5	769.68	761.90	643.99	701.52	39.22						
G:F, g/g						0.763	0.222	<0.0001	0.353	0.376	0.270
Week 1	0.457	0.426	0.867	0.369	0.12						
Week 2	0.727	0.767	0.483	0.659	0.12						
Week 3	0.854	0.759	0.902	0.833	0.12						
Week 4	0.607	0.638	0.641	0.681	0.12						
Week 5	0.723	0.730	0.679	0.673	0.12						

Figure 1. Stool scores per pen each week of the five-week nursery trial. The severity of diarrhea in each pen was scored on a scale of one to five: zero, dry, hard, well-formed feces; one, soft but formed feces; two, pasty feces green or brown in color; three, viscous feces in light color, episodic; four, fluid feces in light color; five, watery feces. This score will be an average of the pen. Bars represent means ± SEM. *P*-values for the main effects of diet, enrichment, time as week and the interactions are reported. Diet P = 0.151; Enrich *P* = 0.783; Day *P* < 0.0001; Diet*Enrich *P* = 0.582; Diet*Day *P* < 0.0001; Enrich*Day *P* = 0.02; Diet*Enrich*Day *P* < 0.0001.

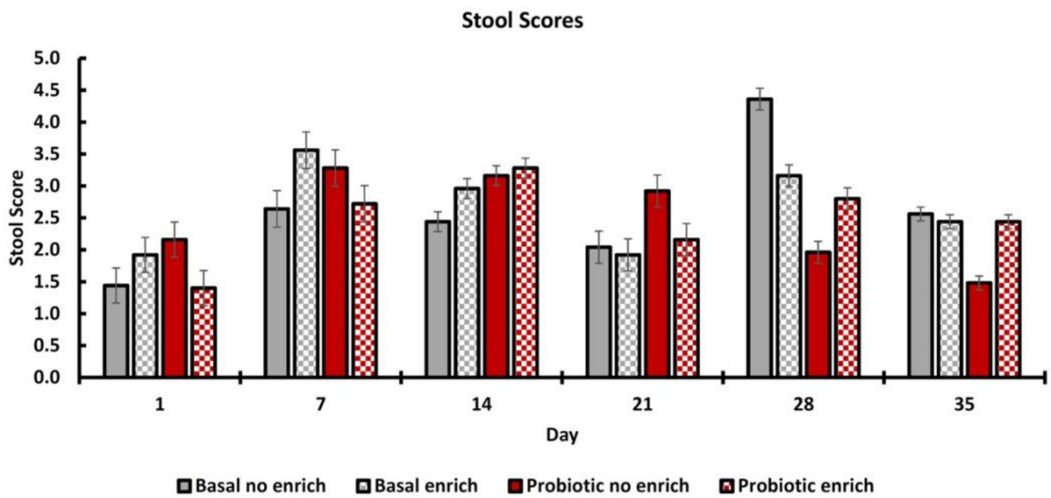
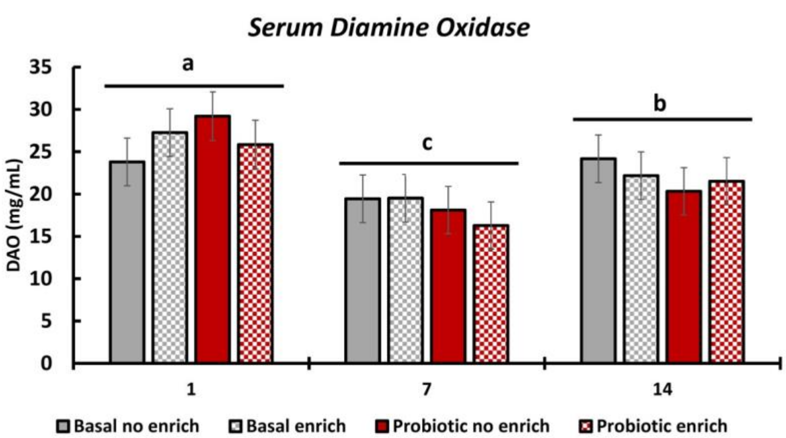


Figure 2. Serum diamine oxidase (DAO). Serum DAO was measured as an marker of oxidative stress. Bars represent means ± SEM. *P*-values for the main effects of diet, enrichment, time as week and the interactions are reported. Diet *P* = 0.67; Enrich *P* = 0.840; Day *P* < 0.0001; Diet*Enrich *P* = 0.647; Diet*Day *P* = 0.365; Enrich*Day *P* = 0.963.



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